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AWARD NUMBER: W81XWH-04-1-0060

TITLE: Development of Immortalized Cell Lines from Hereditary Prostate Cancer Families

PRINCIPAL INVESTIGATOR: Janet Stanford, Ph.D.
Elaine Ostrander, Ph.D.

CONTRACTING ORGANIZATION: Fred Hutchinson Cancer Research Center
Seattle, Washington 98109-1024

REPORT DATE: September 2006

TYPE OF REPORT: Final Addendum

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-09-2006	Final Addendum	15 Feb 2004 – 15 Aug 2006

4. TITLE AND SUBTITLE

Development of Immortalized Cell Lines from Hereditary Prostate Cancer Families

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0060

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Janet Stanford, Ph.D. and Elaine Ostrander, Ph.D.

E-Mail: gmail@fhcrc.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
Seattle, Washington 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer is a significant cause of morbidity and mortality in the United States. Several types of epidemiological studies confirm the existence of a genetic component to prostate cancer etiology. Hereditary prostate cancer (HPC) genes are predicted to account for about 43% of disease in men • 55, and 9% of total prostate cancer in men diagnosed through age 85. To best utilize the finite amount of DNA in blood samples previously collected from 271 HPC families, we are creating immortalized cell lines. The objective is to generate a long term resource of germline DNA from HPC families for use in mapping gene pathways associated with disease susceptibility.

15. SUBJECT TERMS
Hereditary Prostate Cancer, Germline Mutation, Immortalized Cell Lines, Susceptibility Genes, Genome Wide Scan

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	6	**19b. TELEPHONE NUMBER** *(include area code)*

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INTRODUCTION

We are engaged in a collaborative project using high throughput approaches to identify regions of the human genome containing prostate cancer susceptibility genes. Collection of 271 hereditary prostate cancer (HPC) families that make up the PROstate Cancer Genetics REsearch StudieS (PROGRESS) has been coordinated by Dr. Janet Stanford at the Fred Hutchinson Cancer Research Center (FHCRC). Genotyping of families using microsatellite markers has been shared between Dr. Elaine Ostrander's lab at the FHCRC (now at NHGRI/NIH) and Dr. Lee Hood's lab at the Institute for Systems Biology. Linkage analysis is currently under the direction of Dr. Dan Schaid from the Mayo Clinic. All of these activities have been funded by NIH grants R01CA78836 and R01CA80122 to Drs. Ostrander and Stanford, respectively. In our grant, we proposed to make a long-term resource out of 183 of the 271 families by creating immortalized cell lines from a subset of affected men. This will provide an unlimited amount of DNA for long term linkage analysis, positional cloning and mutational analysis studies.

BODY

The following summarizes the objectives of this grant and the work accomplished by the end of the funding period (8/15/06).

Concept

We will develop a long term resource for mutation detection studies in high risk prostate cancer families by developing Epstein Barr Virus (EBV) immortalized cell lines from buffy coat samples prepared previously. The aim was immortalization of two affected men each from as many of the families as possible. As prostate cancer genes are mapped we will use this DNA resource to check for linkage in our data set. As confirmation of linkage takes place, we will use the DNA for positional cloning. Finally, as true susceptibly genes are identified we will use the resource for mutation scanning.

Objective 1 & 2. Subjects to be Immortalized & Immortalization of Cell Lines and DNA Isolation:

Buffy coats from 1,794 individuals who are part of the PROGRESS data set have been prepared and preserved in liquid nitrogen using the method of Louie and King. This methodology is optimal for preparing and storing buffy coats that are suitable for later immortalization with EBV. In this grant, we proposed to immortalize two men from each of 183 families, selecting the youngest affected male and one randomly selected affected male who is a blood relative of the initial individual.

By the end of the funding period, 220 cell lines were generated from 102 of the largest prostate cancer families in PROGRESS. For 22 of the 102 families, only one cell line was successfully generated. While for some key families, such as those that are very large or represent ethnic groups of particular interest, we have targeted more than two individuals for immortalization. Indeed, for one family, 11 cell lines were generated. A total of 275 samples were attempted where 220 cell lines were successful and 55 failed. This corresponds to an overall success rate of 80%, which is slightly below the published success rate of 84%. This might be due to the fact that some of our buffy coats when

generated were not frozen properly and some were badly hemolyzed. DNA samples are targeted for preparation from each of the 220 cell lines using Purgene DNA Purification System (Gentra Systems), where 126 are already completed and 94 will be completed soon.

In total, we established 220 total cell lines, which is less than our original aim to generate 366 cell lines from 188 families in 18 months. The rate of buffy coat transformation initially proposed would correspond to a transformation rate of 20.3 cell lines per month. In the first 7.5 months of the grant period, 100 cell lines were started 74 were transformed. Thus, the rate of successful transformation was an average of 9.9 cell lines per month. During the grant funding period, it was discovered that there is a large amount of variability in the time it takes for the cell lines to transform. The average time for transformation was 46 days with a range of 16 to 103 days. Thus, the major limitation in this project has been time and the incubator space to keep all of these cell lines going.

KEY RESEARCH ACCOMPLISHMENTS

- Selected 275 prepared frozen lymphocyte samples for immortalization.
- Attempted immortalization for all 275 samples selected.
- Successful EBV immortalization for 220 samples.
- A total of 102 families are represented by at least one cell line.
- 80 families are represented by 2 or more cell lines.
- The overall success rate was 80%.
- DNA preps using standard kits are in progress for white cells harvested from the 220 completed cell lines.
- 126 DNA samples are already completed.
- Multiple aliquots of each immortalized cell line have been entered into our database, tracked, frozen, and stored in liquid nitrogen for long term use.

REPORTABLE OUTCOMES

The cell lines developed here and the DNA extracted from them are a long term resource for future endeavors in finding prostate cancer genes. Thus far, they have not directly been used in a study, but will be utilized in future studies for years to come.

CONCLUSIONS

The cell lines developed here will be a long term resource for finding prostate cancer genes. In our own lab, they will be used to fine map regions of interest, to determine the distribution and frequency of mutations developed by others, and to undertake mutation scanning of candidate genes from loci derived from our own genome scan. The data resulting from all of these experiments will benefit the community as a whole as it is shared with our colleagues in the International Consortium of Prostate Cancer Genetics (ICPCG). The ICPCG is comprised of investigators from over a dozen labs around the world that meet twice a year to exchange data, information, resources and reagents, often prepublication, with a goal of facilitating the finding of prostate cancer susceptibility

genes. We are funded by a large NCI grant (P.I. William Isaacs) to facilitate these collaborations, and to undertake meta-analyses. Thus, the funds received from this DOD grant to construct cell lines from our prostate families will benefit the larger collaborative community as well.

REFERENCES

None.

APPENDICES

None.